Environmental Effects on the Growth, Maturation and Physiology in Antarctic Krill (*Euphausia superba*) Over an Annual Cycle: An Experimental Approach

Source: R.King (2010)

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Bachelor of Antarctic Studies with Honours

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June 2010

-- Declaration --

Declaration of Originality:

1. I hereby declare that this thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other tertiary institution, except by way of background information and is duly acknowledged in the thesis.
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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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June 2010

-- Abstract --

Antarctic krill, *Euphausia superba*, is a keystone species in the Antarctic ecosystem, being a major food source for most predators and the target of a substantial fishery. Despite being a critical component in the Southern Ocean, limited information exists on krill growth, maturation and physiology under various light, diet and temperature regimes throughout a full year. Without a comprehensive understanding of these factors, forecasting adaptations in a changing environment is hampered. This study examines the effects of the key environmental parameters (light, food availability and temperature) on growth, maturation and physiology in krill.

Krill were incubated for an annual cycle under natural light (emulating the field environment), and constant food supply and temperature. Krill showed a clear seasonal cycle of growth and maturity in all three temperature treatments (-1°C, 1°C, 3°C). Sex significantly affected the relationship with growth over a year. Overall, females showed higher growth rates than males, and growth rapidly decreased after the peak growth period towards the end of spring. Males peaked in growth and matured earlier than females and decreased growth at a considerably slower rate. Negative growth occurred towards the end of January for both sexes, coinciding with the regression of external sexual characteristics. There was a significant decline in intermoult period (IMP) with increasing temperature and some evidence to suggest that 1°C was optimum for krill growth. The IMP was significantly lower at 1°C than at -1°C, but the difference in growth increment (GI) between the two temperatures was not significantly different, with all growth variables significantly lower at 3°C. For the first time, this study has confirmed that compensation mechanisms do exist between IMP and instantaneous growth rate (IGR) for krill, resulting in short IMP/small IGR to long IMP/large IGR.

Based on external sexual characteristics (female – thelycum; male - petasma), krill exposed to a natural Antarctic light cycle or a fixed light/dark regime, progress under a natural maturation cycle of regression and re-maturation. However, when krill were maintained in complete darkness during sexual regression, the rate of regression accelerated and re-maturation occurred three months earlier in the following season. This flexible maturation cycle in response to conditions of total darkness at the time of regression means that krill can flexibly adjust their seasonal physiological cycle. Overall, light (in this case darkness) appears to be one of the most important factors influencing the krill maturation cycle.

There was a strong significant increasing trend of respiration rates in krill with month in all experimental conditions; natural light cycle versus complete darkness, fed versus starved and different temperature regimes (-1°C, 1°C and 3°C). The interaction of treatment with month, as well as generally the main effect of each treatment, was non-significant. Overall, from this study, it appears that light, food availability and temperature may not be the dominant environmental variables influencing the observed seasonal changes in metabolic rates.

There was no significant difference throughout the year (except February) in total lipid and fatty acid content and composition of immature krill, and also between mature males and females in summer. The lipid and fatty acid concentrations were near depletion in February for all krill, indicating these reserves were possibly used for reproductive purposes rather than as an overwintering source. Mated females were only observed at -1°C in November. Lipid and fatty acid levels were lower in mated compared to un-mated females, indicating utilisation of lipids during the mating process. There was no clear temperature effect on lipid and fatty acid content and composition at the various time points sampled; however, krill at the lower temperature, -1°C, generally contained higher lipid and fatty acid content.

This study has provided a solid basis for understanding the life history of krill over an annual cycle, which will enable more robust modelling for accurate assessments and management for the krill fishery. This research has further helped elucidate the effects of key environmental parameters on the growth, maturation and physiology in krill. It is crucial to expand on this knowledge so as to comprehend seasonal adaptation and survival of krill in a changing habitat, in light of predicted climatic change.

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-- Acknowledgments --

Firstly, I would like to give a special thankyou to my supervisors during this project; Drs So Kawaguchi, Patti Virtue, Peter Nichols and Steve Nicol. I am most grateful for your time, patience, wealth of knowledge and experience, valuable discussions, and constructive feedback on my progress and endless draft material throughout the course of this PhD. Undertaking this PhD has certainly been my most notable achievement to date, and completion was only possible due to your continuous support, direction, mentorship and motivation. Your guidance has really stimulated my interest in krill research and I have enjoyed my time immensely. I have gained so much from your assistance and have learnt a great deal about myself. Thanks to your supervision I have been rewarded with many attributes and skills that I believe I would not have achieved by doing anything else. Additionally, your constant encouragement to apply for funding support has provided me with ample travel opportunities to work and present my research overseas, which is a priceless life experience.

I am appreciative to the staff at the Australian Antarctic Division for the support in the laboratory. I notably like to thank Rob King, Paul Cramp and Andrew Mceldowney for their general support, friendships and contribution and maintenance of the aquarium facilities. I must give a big thankyou to Dr Steve Candy who provided continual time and friendly assistance with statistical advice and application. Dr Paul Thompson also provided time and technical assistance with the use of the FACScan so that I could undertake feeding experiments. I would also like to thank Mina Augerinos and Peter Mansour for their technical assistance and support with lipid analysis at CSIRO. I am also grateful to the captain and crew of RSV *Aurora Australis* for their skillful assistance during the collection of the krill used in this study.

To the many friends that I made along the journey at AAD and UTAS, you made the completion of this PhD possible and an extremely enjoyable experience. Your friendships kept me from going insane during stressful and long hours. You provided a great distraction during work in the office and laboratory with interesting and humorous conversations. Most importantly, I would like to thank all my family, particularly mum and dad, who have provided constant love, support (financial and personal) and encouragement, not only through the course of this PhD, but my whole life. I cannot thank you enough and I am forever grateful. To my closest friends, particularly JJ Zucco (I could not possibly name everyone), I appreciate all the support, reassurance, distractions and endless fun times throughout the last few years.

My time as a PhD student was funded by the Tasmanian Graduate Research Scholarship. My research was also supported by the Australian Government's Cooperative Research Centres Programme through the Antarctic Climate and Ecosystems Cooperative Research Centre (ACE CRC) with a generous annual top-up scholarship. This financial assistance was much needed to complete this PhD and made life a lot easier with healthy and comfortable living.

-- Co-Authorship --

Manuscripts representing the majority of this thesis include:

i. **Brown, M**., Kawaguchi, S., Candy, S. and Virtue, P. (2010). Temperature effects on the growth and maturation of Antarctic krill (*Euphausia superba*). *Deep-Sea Research II*. **57**, 672-682. → **Chapter 2**

ii. **Brown, M**., Kawaguchi, S., King, R., Virtue, P. and. Nicol, S. Flexible adaptation of the seasonal krill maturation cycle in the laboratory. *Submitted to Journal of Plankton Research*. → **Chapter 3**

iii. **Brown, M**., Kawaguchi, S., Candy, S., Yoshida, T., Virtue, P. and Nicol, S. The long-term effect of photoperiod, temperature and feeding regimes on the respiration rates of Antarctic krill (*Euphausia superba*) in the laboratory. *Submitted to Marine Ecology Progress Series*. → **Chapter 4**

iv. **Brown, M**., Virtue, P., Nichols, P., Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on immature Antarctic krill (*Euphausia superba*) over a full year: Lipid and fatty acid content and composition. *Submitted to Comparative Biochemistry and Physiology, Part B*. → **Chapter 5**

v. **Brown, M.,** Virtue, P., Nichols, P. and Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on lipid and fatty acid content and composition with respect to sex and body tissue of Antarctic krill (*Euphausia superba*) in summer. *Submitted to Comparative Biochemistry and Physiology, Part B*. → **Chapter 6**

The following specifies contributions of all authors and supervisors to the above listed chapters/manuscripts:

- The concept and design of this thesis was developed by M. Brown and S. Kawaguchi.
- S. Kawaguchi, P. Virtue, P. Nichols and S. Nicol assisted with the general supervision of this thesis. This included experimental design, general advice, interpretation of data, and proof reading and contributing to the above listed chapters/manuscripts.
- All laboratory experiments and measurements in this thesis were conducted by M. Brown. In Chapter 4, some of the laboratory measurements were performed by T. Yoshida. Laboratory assistance was provided by P. Virtue and P. Nichols with lipid and fatty acid analyses in Chapters 5 and 6.
- Statistical analysis in Chapter 2 and 4 was conducted by S. Candy. Statistics in the remainder of the thesis was performed by M. Brown, with advice from S. Candy.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above submitted peer-reviewed chapters/manuscripts contributing to this thesis.

Supervisor:	Supervisor:	Supervisor:	Supervisor:
Dr. So Kawaguchi	Dr. Patti Virtue	Dr. Peter Nichols	Dr. Steve Nicol

-- Acronyms and Abbreviations --

Definitions of the main acronyms used throughout the thesis:

Acronym	Definition
°C	Degrees Celcius
AA	Arachidonic acid (20:4ω6)
AAD	Australian Antarctic Division
ANOVA	Analysis of variance
BAV	Between-animal variance
CCAMLR	Commission for (and Convention on) the Conservation of Antarctic Marine Living Resources
Chl *a*	Chlorophyll *a*
DAG	Diacylglycerol
DHA	Docosahexaenoic acid (22:6ω3)
DGR	Daily growth rate (mm day^{-1})
DW	Dry weight (mg)
EPA	Eicosapentaenoic acid (20:5ω3)
F	Female
FA	Fatty acid
FAME	Fatty acid methyl esters
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometer
GI	Growth increment in total length (mm)
H1	Holding tank 1
H2	Holding tank 2
HC	Hydrocarbon
hr	Hour
IGR	Instantaneous growth rate (%)
ind	Individual
IMP	Intermoult period (days)
L	Litre
LA	Linoleic acid (18:2ω6)
LMM	Linear mixed model
M	Male
m	Metre
mL	Milli-Litre
month.f	Month as a factor, with a set of integer values ranging between 4-17 (Apr 06 – May 07)
MUFA	Monounsaturated fatty acid
MS	Maturity score
NVE	Night vision equipment
O_2	Oxygen
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RMT	Rectangular mid-water trawl (net)
RSV	Research and scientific vessel

Acronym	Definition
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acid
sp.	Species
TAG	Triacylglycerol
TLC-FID	Thin-layer chromatography-flame ionization detector
TL	Total length of the krill (Standard length 1)
TL.f	Total length as a factor, with classes of <31,31-33,33-35, 35-37, 37-39, >39mm
TSE	Total solvent extract
UL	Uropod length (mm)
µL	Micro-Litre
WAV	Within-animal variance
WE	Wax ester